

GEORGIA INSTITUTE OF TECHNOLOGY
Engineering Experiment Station

PROJECT INITIATION

Date September 23, 1963

Project Title: X-Radiation Effects in Euploid Human Cell Cultures

Project No.: B-258

Project Director: R. H. Fetner

Sponsor: Public Health Service, Bureau of State Service

Effective: 9-1-63 Estimated to run until: 8-31-64

Type agreement: Research Grant No. RH 00035-03

Amount: \$15,529.00

Reports: Short Summary Progress Report to
accompany Renewal application

Contact Person: Dr. Paul F. Hahn
Chief, Research Grants Staff
Division of Radiological Health
Bureau of State Service
Washington 25, D. C.

NOTE: This is a continuation of B-25 238

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GEORGIA INSTITUTE OF TECHNOLOGY
Engineering Experiment Station

PROJECT TERMINATION

Date 10-3-64

PROJECT TITLE: X-radiation Effects in Euploid Human Cell Cultures

PROJECT NO: B-258

PROJECT DIRECTOR: R. H. Petner

SPONSOR: Public Health Service, Bureau of State Services

TERMINATION EFFECTIVE: 3-31-64

CHARGES SHOULD CLEAR ACCOUNTING BY: 9-30-64

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GEORGIA INSTITUTE OF TECHNOLOGY
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PROJECT INITIATION

Date August 25, 1964

Project Title: X-Radiation Effects in Euploid Human Cell Cultures

Project No.: B-274

Project Director: R. H. Fetner

Sponsor: Public Health Service

Effective: 9-1-64 Estimated to run until: 8-31-65

Type agreement: Grant No. RH 00035-04

Amount: \$16,347

Reports: Final Report

Contact Person: Dr. Paul F. Hahn, Chief
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Engineering Experiment Station

PROJECT TERMINATION

Date January 8 16, 1968

PROJECT TITLE: X-Radiation Effects in Euploid Human Cell Cultures

PROJECT NO: B-274

PROJECT DIRECTOR: R. H. Fetner

SPONSOR: Public Health Service

TERMINATION EFFECTIVE: 8-31-65

CHARGES SHOULD CLEAR ACCOUNTING BY: All acceptable charges have cleared

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*Reports
B-274*

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ENGINEERING EXPERIMENT STATION
OF THE GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA

Final Report for period September 1, 1964 to November 15, 1965.

X-Radiation Effect in Euploid Human Cell Cultures

by

Robert H. Fetner

Grant in aid RH 00035-03

From Division of Radiological Health

Public Health Service

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SUMMARY STATEMENT

Some of the effects of 250 KV x-rays on adapted human cell lines were studied. In the initial phase the frequency of various anaphase abnormalities were investigated and multipolar mitosis were singled out as a single type of abnormality of particular interest. The frequency of multipolar mitosis in the KB cell line was determined over an extended period of time (14 months). The frequency of multipolar anaphases was found to be $.038 \pm .007$ and to remain constant within the limits of experimental error. The effects of various doses of 250 KV x-rays on the frequency of multipolarity was investigated and was found to increase as a function dose. These results have been published and the biological implications of these phenomena were discussed.

In the next phase of the investigation the feasibility of using cell size (volume) as a cell line specific parameter was studied. A general hypothesis was made of the following relationship:

$$\begin{array}{ccccc} \text{Chromosome} & & & & \\ \text{number} & \sim & \text{Nuclear volume} & \sim & \text{Cell size} \end{array}$$

Electronic grating devices are now available (Coulter counter) which make cell population sizing both rapid and highly quantative. As a first step, four different cell lines were investigated over an extended period of time. There was considerable variation within cell lines over this time interval, however, two of the cell lines (KB and HeLa) were significantly different and could be distinguished. The analysis of these data is complicated by the fact that the frequency distribution of these cell lines is a log-normal distribution. While the significance of this distribution has been extensively discussed we are inclined to agree with Bonner and Eden (1) "that the simplest and most straightforward presentation of the data is the most useful." In general,

distributions were apparently similar (slopes of probit regression lines). If a comparison is to be made either within a cell line or between cell lines some measure of central tendency would seem to be indicated as the standard of comparison. This poses a problem. Remembering that the ultimate objective is to relate size to chromosome number, the mode or the median may be indicated. The mode is most frequently used when determination of the number of chromosomes per cell are made from cytological counts, however, in the sizing work the class frequencies were not of the right magnitude. For this reason the median was used as a standard of comparison.

A size frequency distribution was also made of isolated nuclei from the different cell lines. Although these data are complicated by the presence of cell debris, the results conform to the whole cell data in that the same kind of size distribution is obtained and the relative median nuclear volumes are different between the HeLa and KB cell lines.

In the two cell lines which had the greatest differences in median cell size, KB and HeLa, chromosome counts were made on a number of metaphase cells and the results presented as frequency histograms. The frequency distribution of chromosome numbers is similar to both the median cell size and the cell volume distributions. The cell line with the smaller median cell size and the nuclear size has the lowest median chromosome number. Karyotype analysis are time consuming and are subject to a number of human errors. They particularly suffer from lack of quantitation.

In the final phase of this work cell size distributions were made on cell lines after various doses of x-rays for various increments of time after

(1) J. T. Bonner and E. Eden, Exp. Cell Res. 11 (1956).

irradiation. The effects of irradiation demonstrate that after irradiation there is an increase in the mean cell size. This is dose dependent and suggests that it is an expression of the effect of mitotic inhibition, or removal of cells in their early growth stage (telephase-interphase) and subsequent increase of cells in later stages of mitosis.

LIST OF RESULTS

In the following list of results those items which have been covered in previous progress reports and pertain to work completed on this grant prior to September 1, 1965 will be summarized.

1. Multipolar mitosis in the KB (Eagle) cell line and its increased frequency as a function of 250 KV x irradiation.

This was the first phase of the program which was completed and furnished the data for a published report (Exp. Cell Res. 37, 429-439).

The frequency of multipolar anaphases in the KB human cell line were found to be 0.038 ± 0.007 when studied over a period of 14 months. Multipolarity was about evenly divided between tripolar divisions and tetapolar divisions with a much smaller frequency of cells observed with greater polarity.

Irradiation with 250 KV x-rays increased the frequency of multipolarity observed. A high positive linear correlation was found between the maximum frequency of induced multipolarity and radiation dose. About 400 r would produce 50 per cent multipolarity and 100 per cent multipolar divisions would theoretically result from 800 r. If the "survival curve" (dipolar cells) is considered an exponential function of radiation dose, then the mean lethal dose would be about 590 r. These data indicate that radiation induced multipolarity is the result of damage to a sensitive volume (target). The dose required to double the background frequency of multipolarity suggest this primary target has approximately the same sensitivity (or size according to the target theory of Lea) as gene mutations. A single or functionally double centriole does not appear to satisfy the requirements for the target, because there was neither a sequential appearance of tripolar and tetapolar spindles

after irradiation nor was there a differential frequency of occurrence between the two kinds of multipolarity.

2. Size frequency distributions of four adapted human cell lines.

Cells in the logarithmic growth phase from the four cell lines were harvested by a 5 minute exposure to 10 ml of 0.5 per cent trypsin in a calcium and magnesium-free saline and then aspirated through a 22 gauge needle until microscopic observation indicated a single cell suspension. This procedure was usually completed with less than 10 aspirations. The resulting single cell suspension was brought up to a volume of 70 ml with .85 per cent sodium chloride solution. Most of this total volume was used during the size analysis because a separate run is made at each of the window apertures and usually 17 or 18 of successive determinations were necessary for a particular cell line. Each count required 0.5 ml of sample and duplicate runs were made for each sample. The measures of central tendency obtained from 10 replicate size distributions over a 12 to 16 week period for two of the four adapted cell lines are presented in Table I. The mean cell size of the KB and HeLa cell populations were significantly different; the difference between the two means was almost seven times as great as the standard error of the difference. The difference between the medians was almost 6 times the standard error of the difference. The average coefficient of variation of the two cell populations are also significantly different, which indicates that the variance on a logarithmic scale of the two populations will also be different.

The mean probit regression line of 10 successive runs for the four cell lines are shown in Figure 2-5, twice the standard errors are represented by tick marks. If we express the size scale in logarithmic units as in Figure 6 it apparently shows an excellent fit as a log normal type of distribution.

It is apparent from our data that the size distribution of these two adapted cell lines (KB and HeLa) remains constant over an extended period of time and, therefore, may represent an important characteristic useful for distinguishing differences between different cell lines, or in detecting effects of various agents, such as radiation.

3. Isolated nuclear size distributions of human cell lines.

In an attempt to correlate nuclear size and cell size in the two cell lines which showed the greatest size difference (KB and HeLa) various methods were investigated for isolation of nuclei. Tween 80 and 0.1 N Citric Acid gave the best results and of the two the citric acid method gave the cleanest nuclei as observed under the microscope.

Nuclei were obtained by replacing the media on actively growing cell cultures with the citric acid and then vigorously agitating the bottle until the cells were freed from the glass. The cell suspension was then aspirated through a 25 gauge needle ten times. The nuclei suspension was then diluted into the appropriate concentration range ($20-180 \times 10^4$ particles ml) and then sized on the Coulter counter.

The mean nuclear volume of the two cell lines is presented in Table II.

4. Distribution of chromosome number per cell in KB and HeLa cell populations.

In an attempt to establish a relationship between: Chromosome number nuclear size - cell size, karyotype enumerations were made from metaphase cells of the HeLa and KB cell line (Figure 9 and 10).

The mean number of chromosomes per cell is presented in Table III. It is recognized that the mean number of chromosomes per cell is not the usual way in which karyotype analysis are presented, the mode being more common, however, to facilitate a comparison with the other data on size the mean was

selected. In general the relationship between chromosome number - nuclear volume and cell volume which was originally postulated, hold up in these two cell lines. If this is expressed as a $\frac{\text{HeLa}}{\text{KB}}$ ratio then for mean cell volume it is .787, for mean nuclear volume it is .731 and for mean chromosome number per cell .911. There is a much better agreement between cell volume and nuclear volume, but this may be a result of the larger sample as compared with the chromosome analysis.

5. Effects of radiation on size distribution of KB cell line.

Actively growing replicate cultures of KB cell lines were irradiated with various doses of 250 KV x-rays and then periodically some of the cultures were harvested and sized (see section 2 for method). Figures 9, 10 and 11 show the size distribution after 100, 200 and 400 r at 24, 48 and 72 hours past irradiation. In general the effects of irradiation at these doses is manifest by an increase in the size distribution. This can be explained by mitotoc inhibition which removes the cells in early growth state (telephase-interphase) and accumulates cells in later growth stages. More work will be performed in this area in the immediate future.

TABLE I. Measures of central tendency for size distributions of 4 adapted human cell lines.

	MEAN (μ^3)	STANDARD DEVIATION (μ^3)	MEDIAN (μ^3)	MODE (μ^3)	COEFFICIENT OF VARIATION %	SKEWNESS	NO. REPLICATES
KB	4241 \pm 109	1313 \pm 14	4049 \pm 121	3657 \pm 154	31.12 \pm 2.41	+.4438 \pm .0602	10
Hela, S-3	3339 \pm 72	1257 \pm 18	3079 \pm 68	2747 \pm 95	37.81 \pm .64	+.4715 \pm .0499	10
HEp	3822 \pm 65	1274 \pm 14	3658 \pm 101	3228 \pm 93	33.40 \pm .51	+.4689 \pm .0487	12
FL	3385 \pm 81	1192 \pm 17	3144 \pm 45	2761 \pm 101	35.27 \pm .51	+.5254 \pm .0569	11

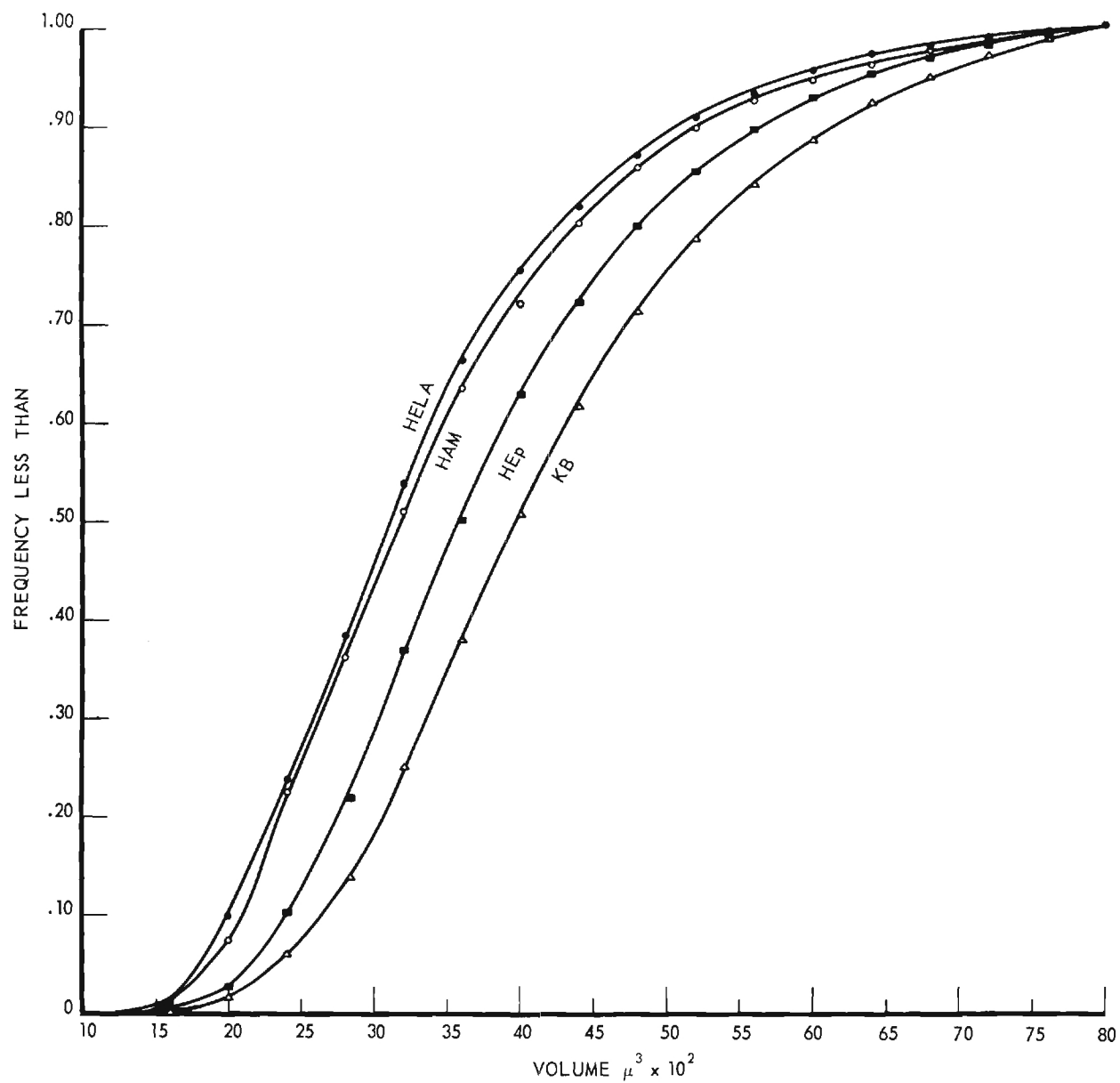


FIGURE 1. Cumulative size frequencies of 4 adapted human cell lines.

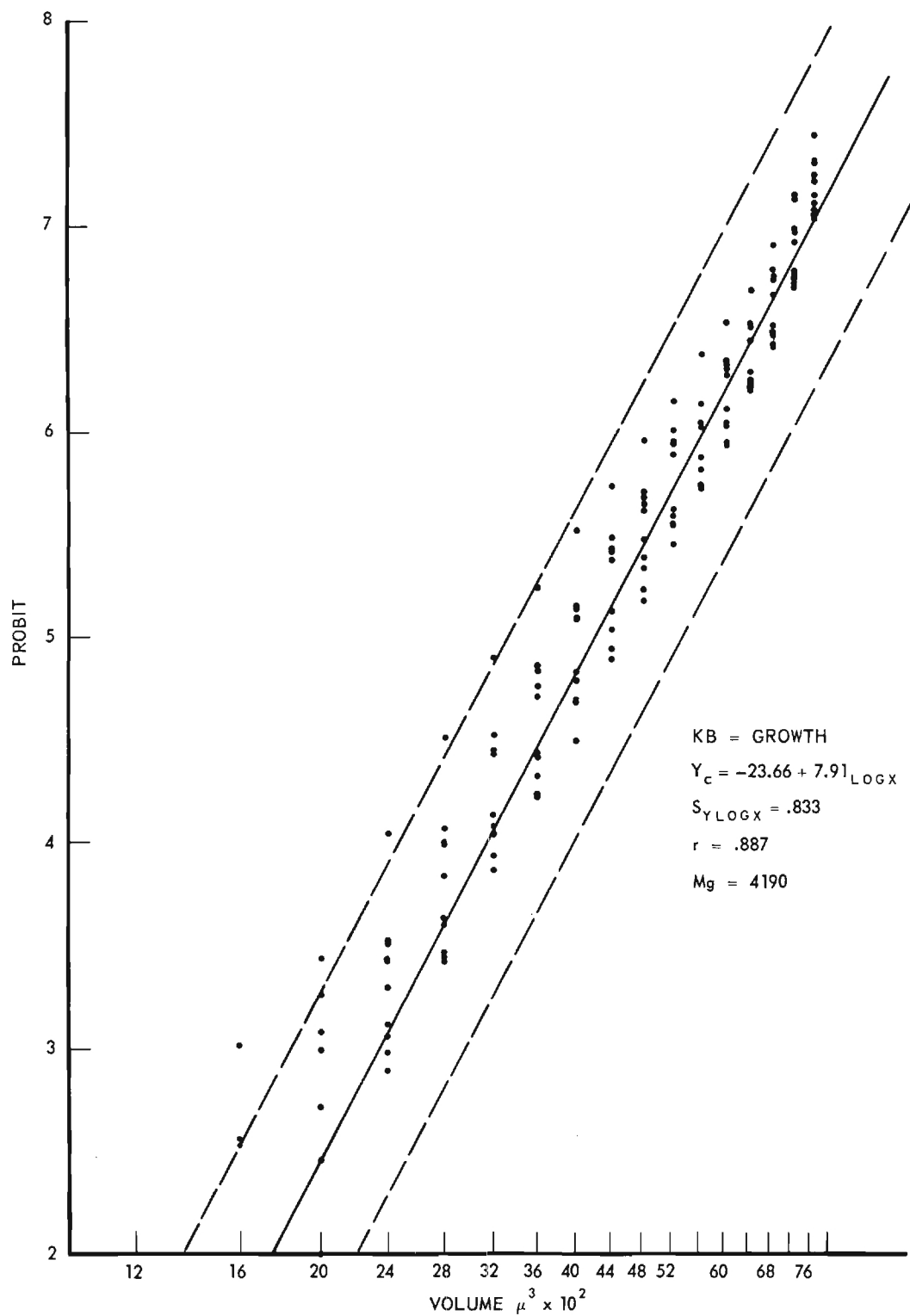


FIGURE 2. Probit regression of size distribution in KB cell line.

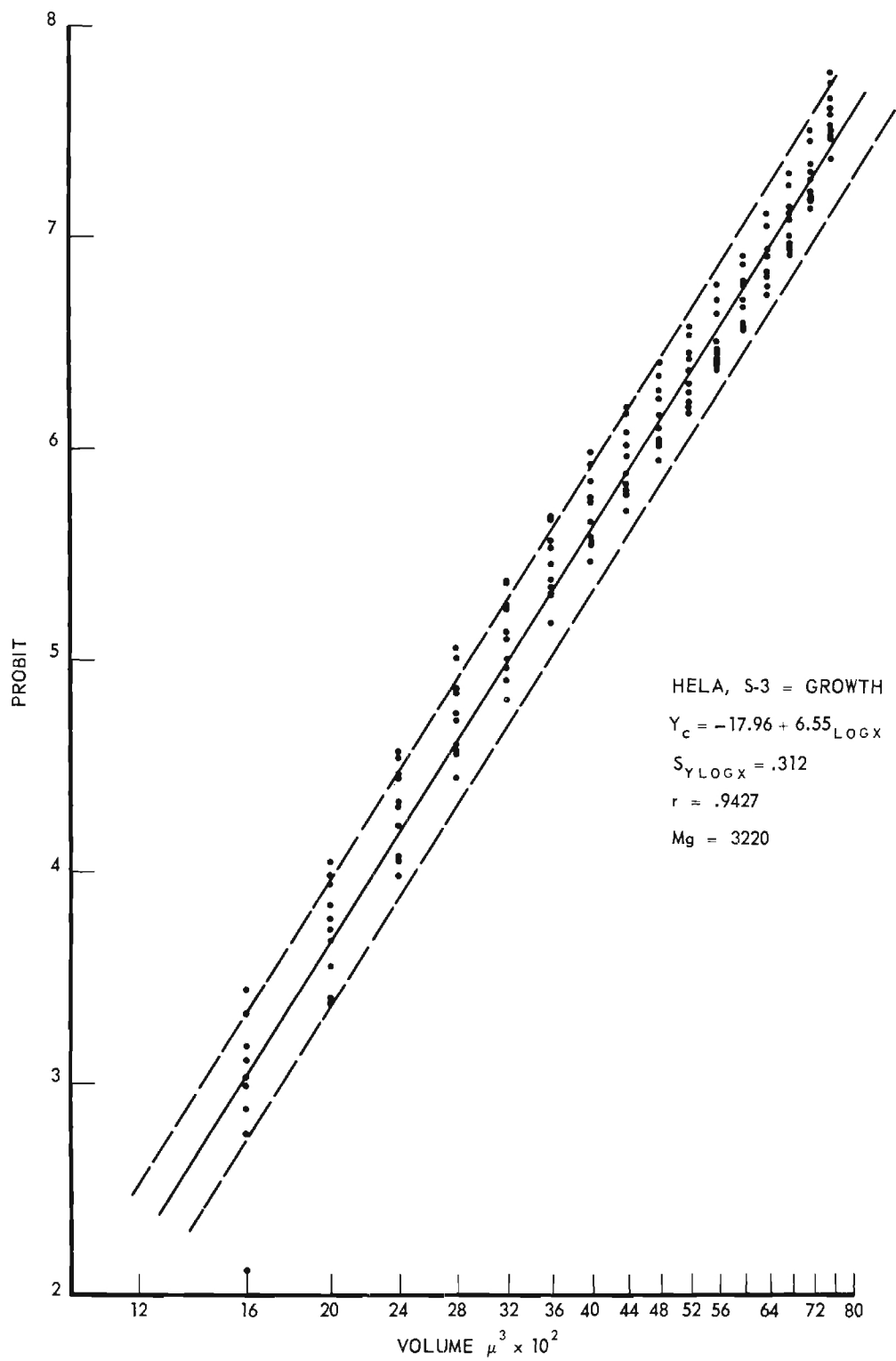


FIGURE 3. Probit regression of size distribution of HeLa cell line.

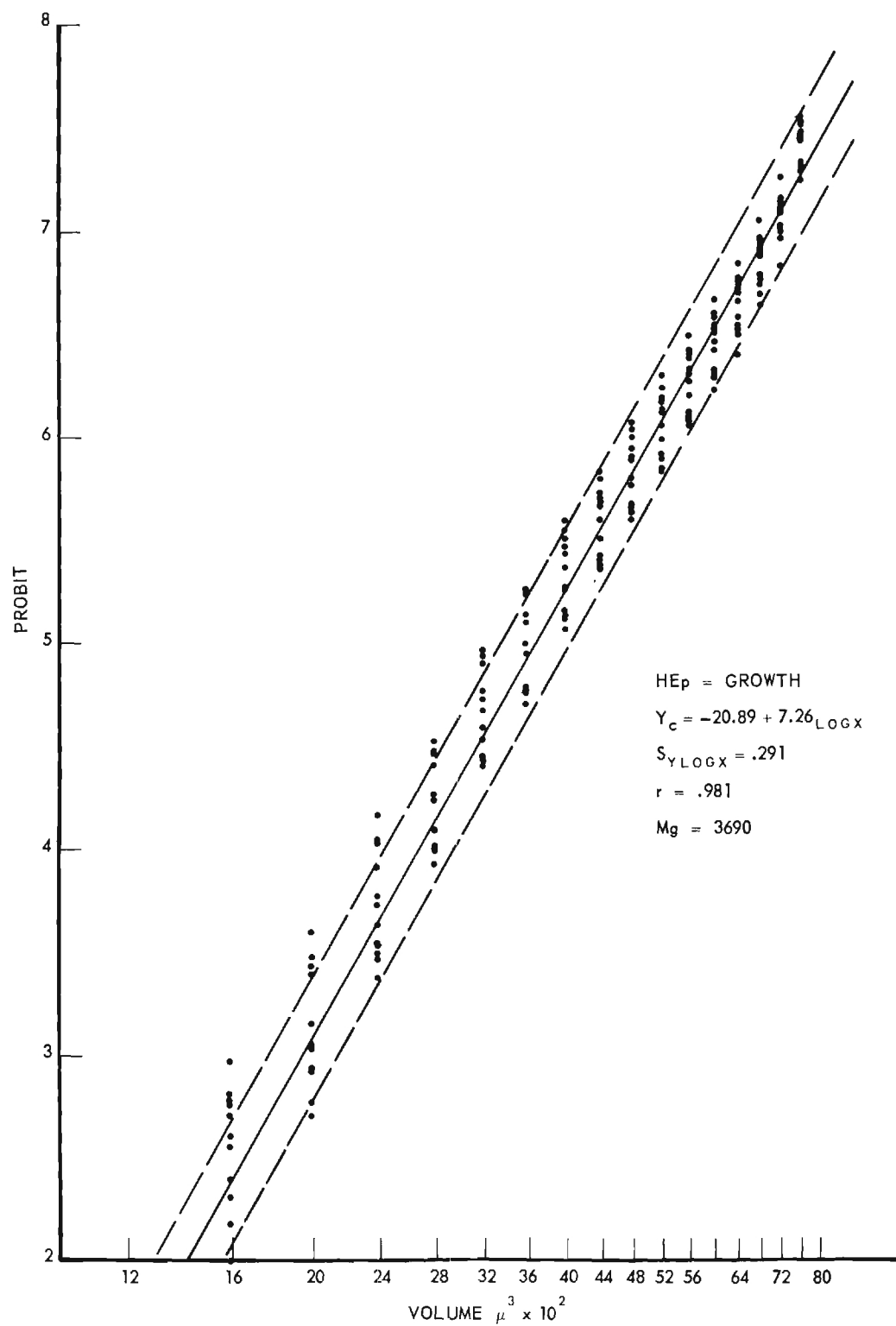


FIGURE 4. Probit regression of size distribution in HEp cell line.

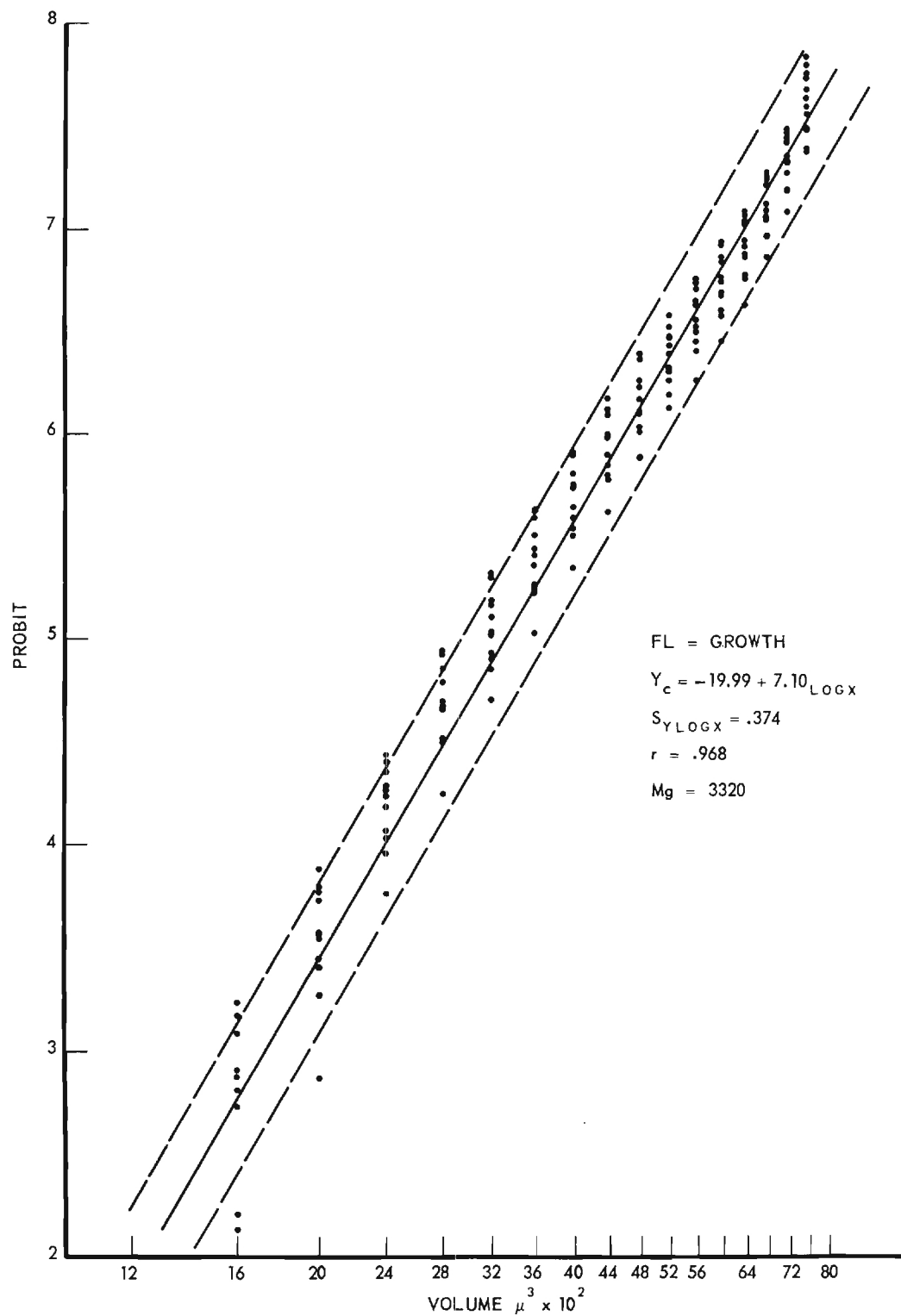


FIGURE 5. Probit regression of size distribution of FL cell line.

TABLE II

Means and standard deviations of the volumes of isolated
nuclei of KB and HeLa cell lines

Cell line	Mean (μ^3)	S.D.	Number of replicate distributions
KB	150.5	4.2	5
HeLa	110.0	0.7	5

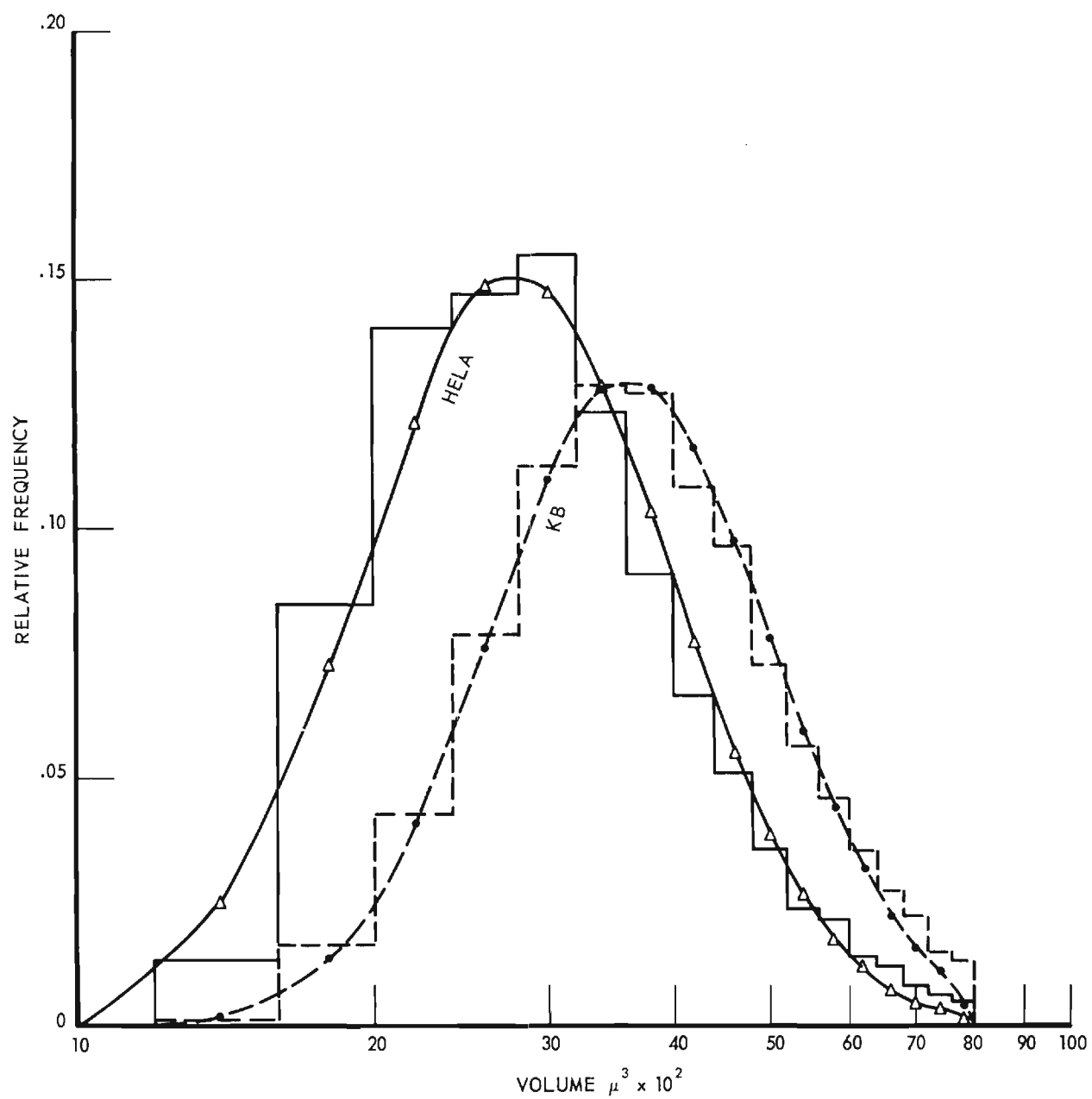


FIGURE 6. Relative frequency. (10 replicates) of KB and HeLa cell lines as a function of size on a logarithmic scale.

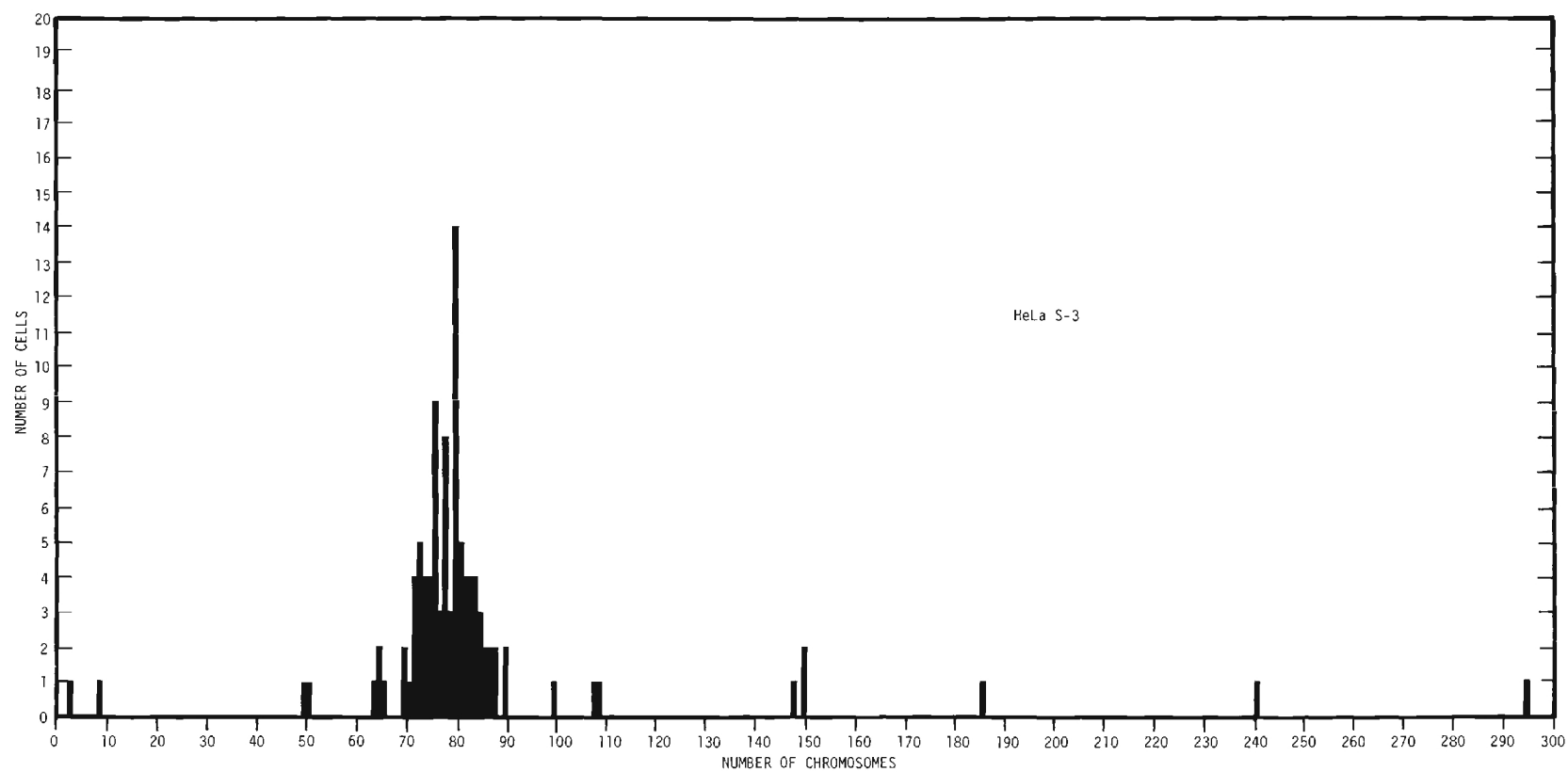


FIGURE 7. Chromosome number per cell frequency histogram of HeLa S-3 cell line.

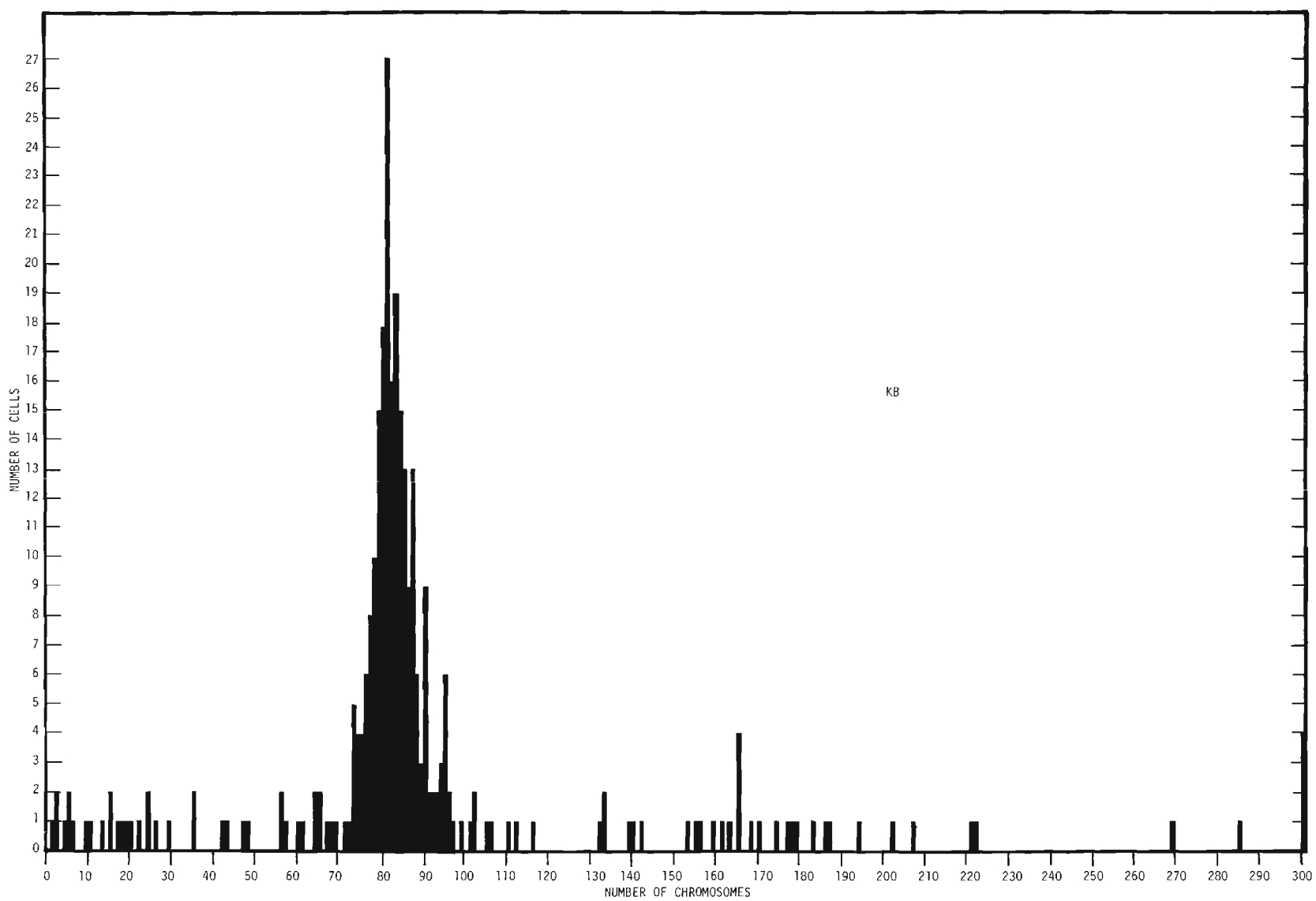


FIGURE 8. Chromosome number per cell frequency histogram of KB cell line.

TABLE III

Mean number of chromosomes per cell
in KB and HeLa cell line

Cell line	Mean no. chromosome per cell	No. of cells counted
KB	91.8	305
HeLa	83.6	101

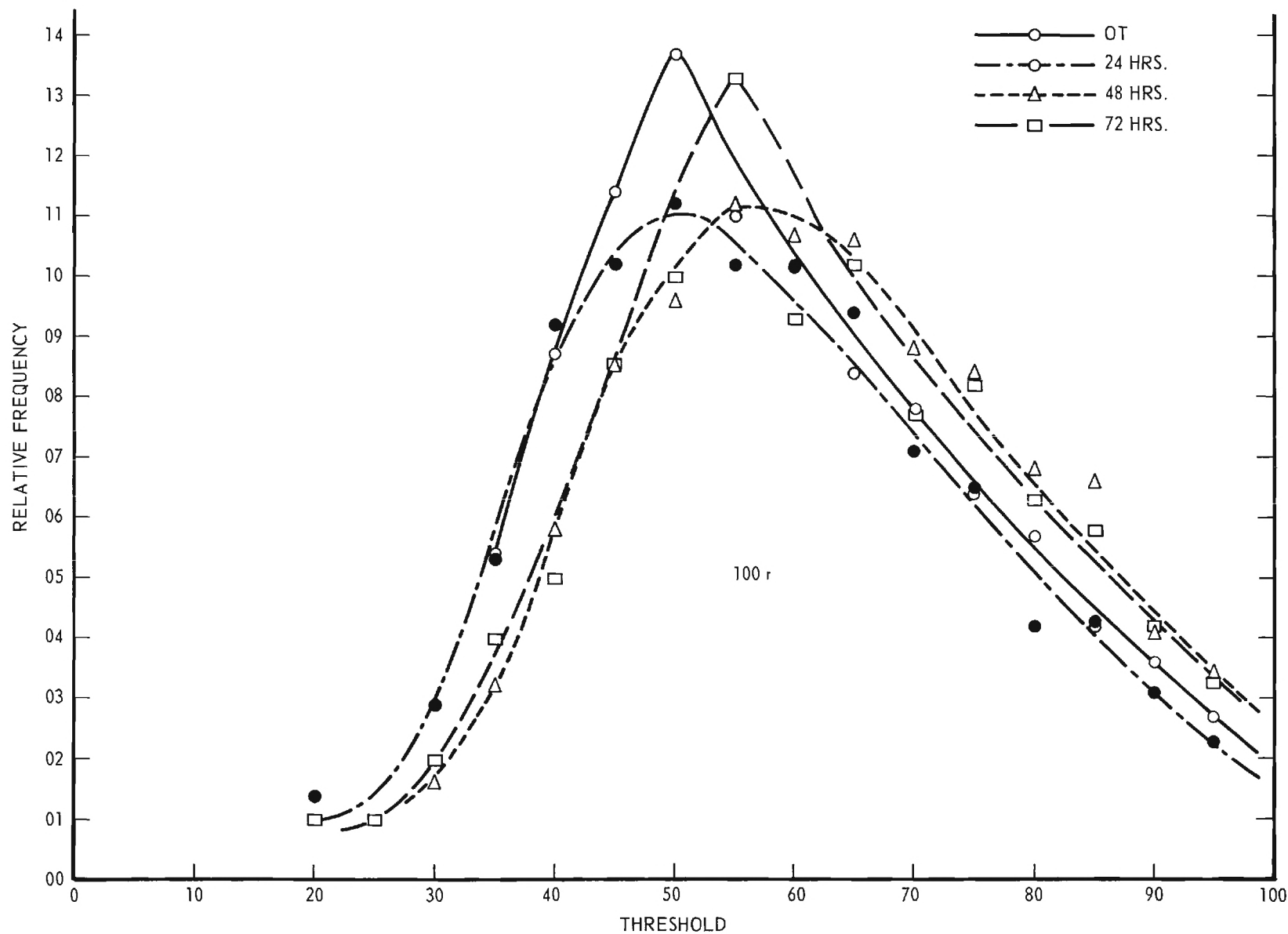


FIGURE 9. Frequency distribution of cell volumes in KB cell line at various increments of time after irradiation with 100 r.

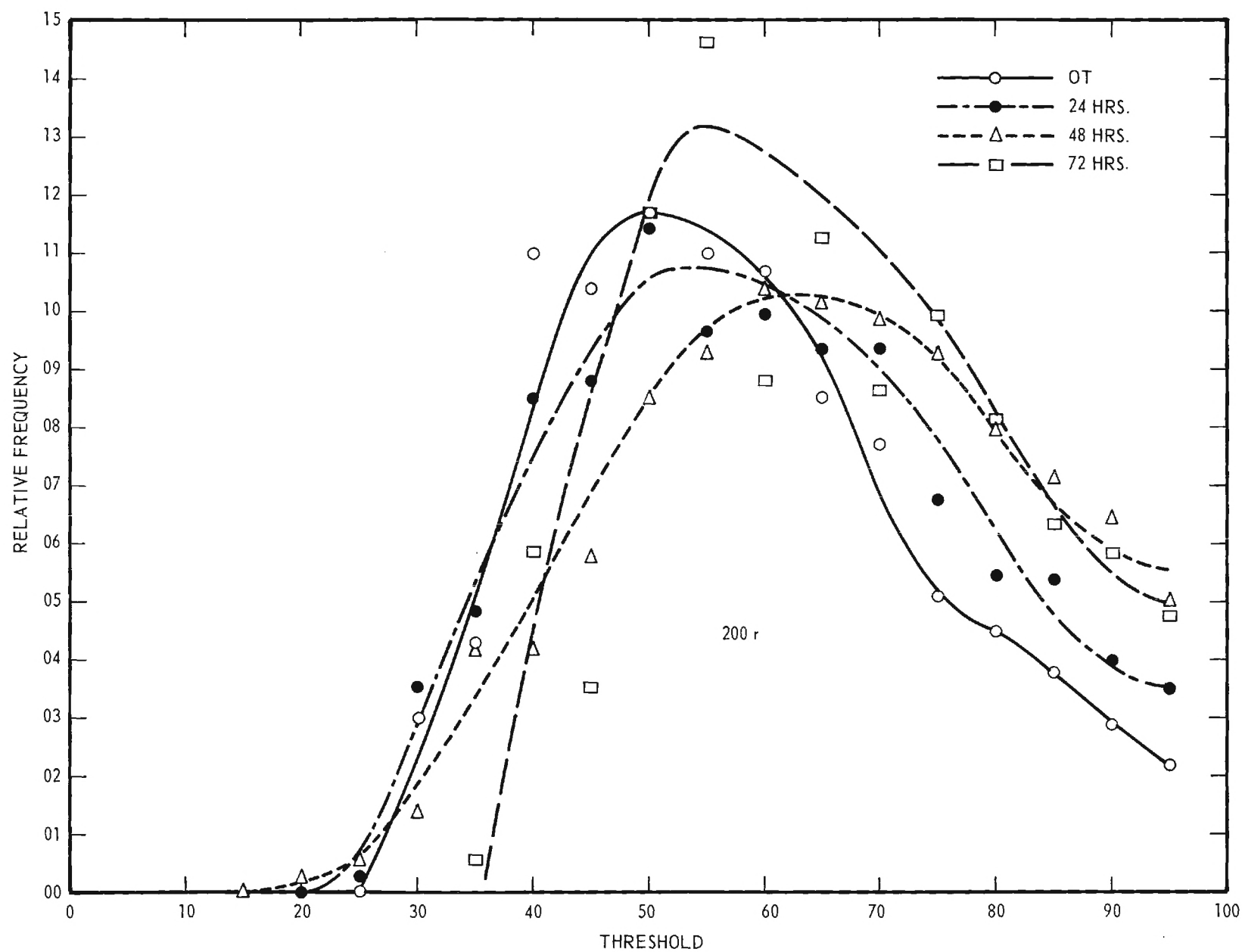


FIGURE 10. Frequency distribution of cell volumes in KB cell line at various increments of time after 200 r.

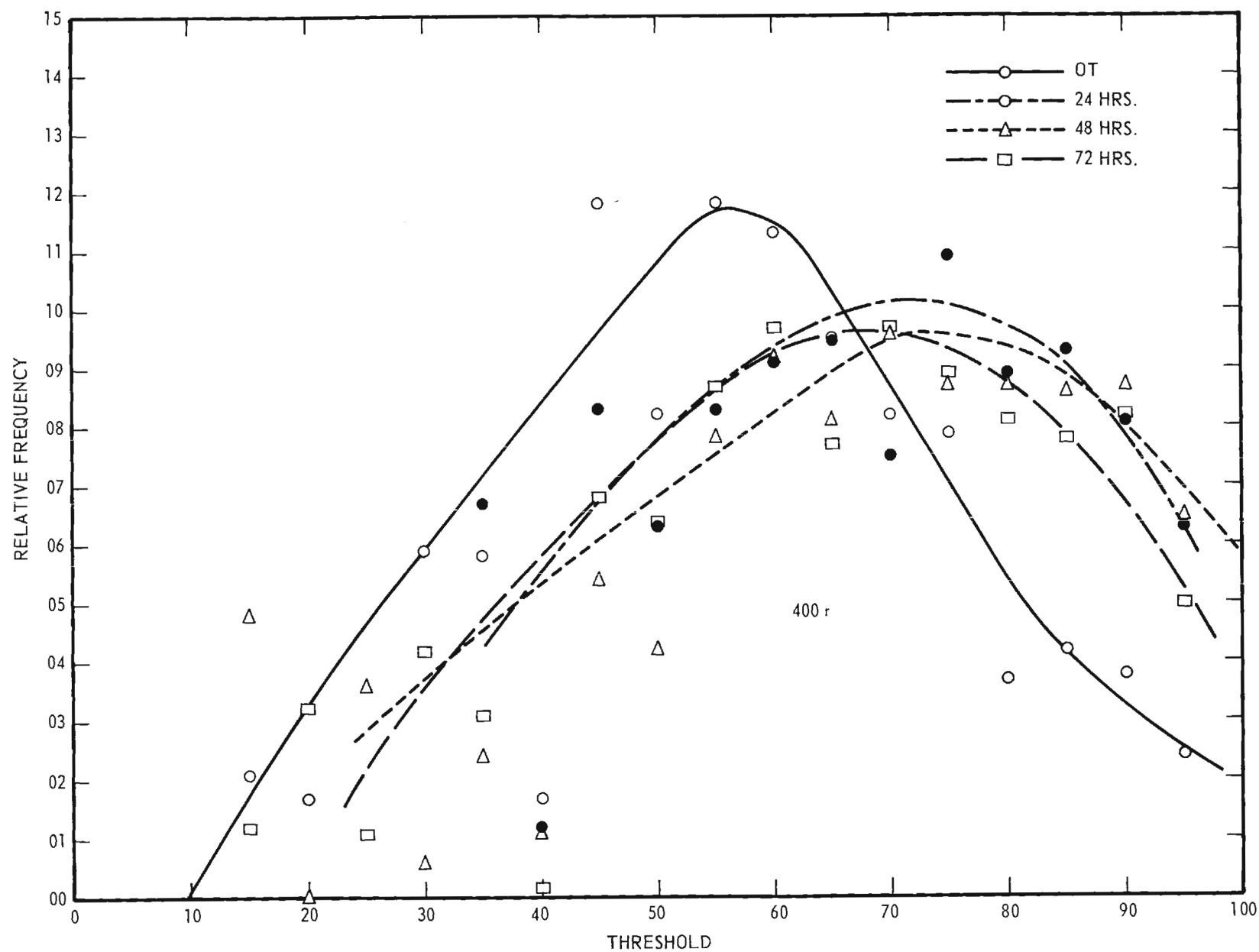


FIGURE 11. Frequency distribution of cell volumes of KB cell line at various increments of time after 400 r.

PUBLICATIONS BASED ON THESE DATA

1. Articles published:

Multipolar mitosis in the KB (Eagle) human cell line and its increased frequency as a function of 250 KV x irradiation. Exp. Cell Research 37, 429-439 (1965).

2. Articles in preparation:

Size distributions in KB (Eagle), HeLa (Gey), HEp (Toolon) and FI (Fogh) cell lines over an extended period of culture.

The relationship between chromosome number - nuclear size - and cell size in KB and HeLa cell lines.